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A CHARACTERISTIC MEMBRANE PROTEIN OF LIVER PEROXISOMES INDUCIBLE BY CLOFIBRATE

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Summary

In sodium dodecyl sulfate polyacrylamide electrophoresis the membranes of rat liver peroxisomes show nine main protein bands (40 000—100 000 dalton); the 40 000-dalton polypeptide cannot be resolved from the membrane by deoxycholate. Treatment of the rats with clofibrate largely increases this protein and another one (about 80 000 dalton) in the peroxisomal but not in the endoplasmic reticulum membrane. Proliferation of peroxisomes seems to be connected with the insertion of specific proteins into the membrane.

The peroxisomal membrane presumably is an outgrowth of the endoplasmic reticulum; this is suggested by morphological evidence [1, 2], lipid analyses and detection of NADH—cytochrome *c* reductase and cytochrome b_5 in either membrane [3, 4]. On the other hand, peroxisomes are enzymatically different from endoplasmic reticulum: beyond their specific markers catalase and H_2O_2 producing oxidases they possess other enzyme activities not found in the endoplasmic reticulum, such as fatty acid β-oxidation [5, 6] The transport of catalase and urate oxidase, two non-membrane enzymes, through the peroxisomal membrane by post-translational transfer has been described recently [7]. A necessary prerequisite for this step may be the co-translational insertion of typical intrinsic proteins which render the peroxisomal membrane an individual entity different from endoplasmic reticulum. Thus an examination of membrane proteins under normal and proliferative conditions seemed desirable. Rat liver under the prolonged influence of clofibrate (ethyl-α-(*p*-chlorophenoxy)isobutyrate), a hypolipidemic drug inducing a well-described increase in the number of peroxisomes [8—10], provides a suitable model for proliferation of peroxisomal membrane. Sodium dodecyl

sulfate (SDS)-polyacrylamide electrophoresis was used for separating proteins of the solubilized membrane preparations.

From the liver of male rats, pretreated with Triton WR-1339 (85 mg/100 g body weight) 3 days before killing, the 'light mitochondrial' fraction was sedimented by differential centrifugation [11] and applied to a discontinuous sucrose gradient (1.16/1.18/1.21/1.26 g/cm^3); the peroxisomes banding at the 1.21/1.26 g/cm^3 interface were distintegrated by dilution with 2 vols. of 10 mM sodium pyrophosphate, pH 9.0, at room temperature for 4 h [12]. The peroxisomal membranes were collected in a second discontinuous gradient (1.13/1.18/1.26 g/cm^3) between the two upper layers [5]. From the supernatant of the light mitochondrial fraction crude microsomes were pelleted (1 h, 100 000 × *g*), resuspended in 0.88 M sucrose and centrifuged for 8 h at 40 000 rev./min on a layer of 1.31 M sucrose [12, 13]. The smooth endoplasmic reticulum fraction, banding at the interface, was diluted with 0.15 M Tris-HCl, pH 8.5, to 0.5 mg protein/ml and purified by pelleting twice at 100 000 × *g* for 90 min. Detergent-extractable proteins were solubilized by treatment of the membrane fractions with 1% deoxycholate in 50 mM Tricine, pH 7.5, for 2 h. Residual membrane was resedimented for 1 h at 300 000 × *g*. The supernatant was dialyzed and lyophilized. For electrophoresis the membrane preparations were solubilized for 5 min at 100°C with SDS mercaptoethanol urea [14] ; electrophoresis was carried out in polyacrylamide-SDS gels according to Zahler [15]. Gels were stained with amido black and subjected to optical densitometry. Molecular weights were estimated with appropriate standard proteins [14]. Glycoproteins were detected by periodic acid—Schiff reagent staining [16].

Nine main protein bands ranging between the molecular weights of approx. 40 000 and 100 000 are observed in peroxisomal membranes (Fig. 1, trace 1). In the endoplasmic reticulum pattern the fastest of the peroxisomal main bands is rather weak, whereas two stronger bands in the range below 40 000 daltons are seen (Fig. 1, trace 5). The characteristic 40 000-peroxisomal component which contains carbohydrate (detected by the periodic acid-stain [16]) cannot be dissociated appreciably from the membrane by deoxycholate whereas the heavier components are more-or-less extractable (Fig. 1, traces 3 and 4). Application of clofibrate produces significant changes, especially in the peroxisomal membrane (Fig. 1, traces 2 and 6). Most conspiciously, the

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Fig. 1. SDS-polyacrylamide electrophoresis of rat liver peroxisomal and microsomal membranes.

1. Peroxisomal membranes, control;

2. Peroxisomal membranes of rats fed for 3 weeks a diet containing 0.75% clofibrate;

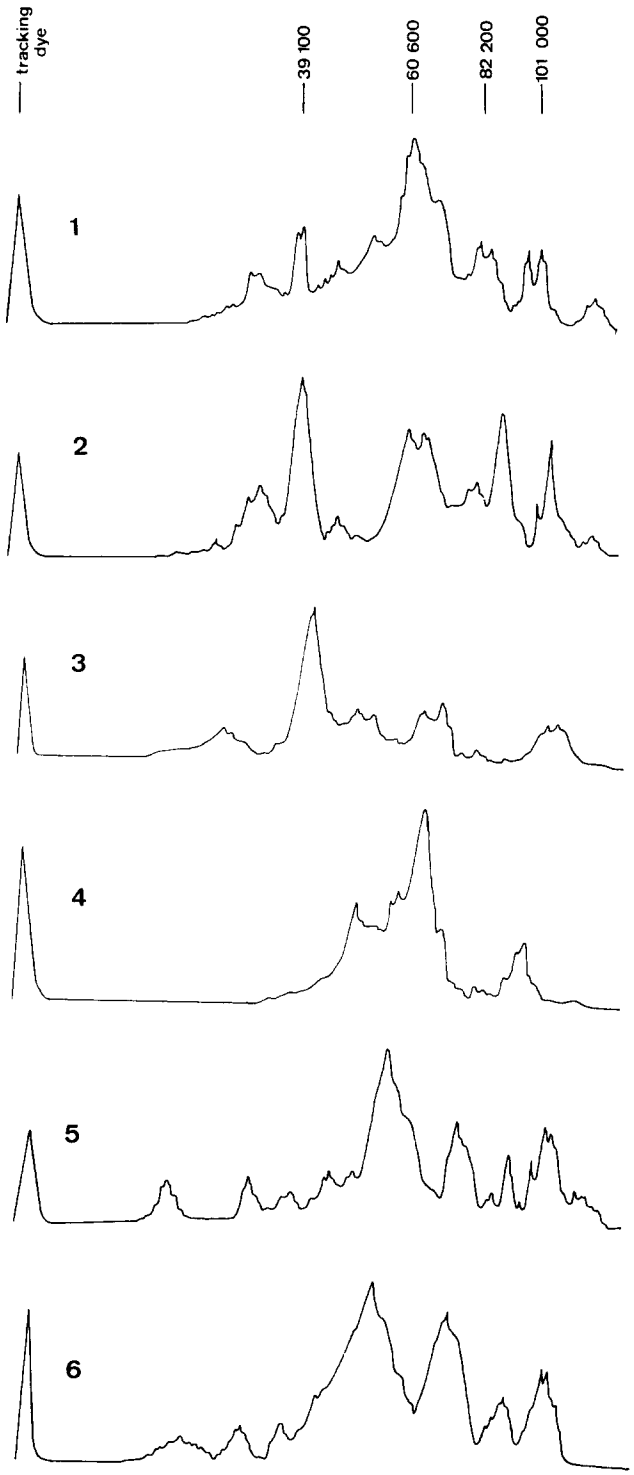
3. Peroxisomal membranes extracted with deoxycholate;

4. Peroxisomal membranes, lyophilized deoxycholate extract;

5. Smooth endoplasmic reticulum membranes, control;

6. Smooth endoplasmic reticulum membranes after clofibrate feeding;

Start: In the right. Ordinate: relative light absorption. Tracking dye: Bromophenol blue. Figures in trace 1: approximate molecular weights.


tracking dye
39 100
60 600
82 200
101 000
1
2
3
4
5
6

40 000-protein and another component with about twice this molecular weight are raised. The latter component might be identical with the peroxisome proliferation associated polypeptide which, after application of hypolipidemic drugs, was found to be increased in crude cell fractions [17]. Our findings are compatible with the view that the independent existence of the peroxisomal membrane begins from the emergence of characteristic intrinsic proteins.

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